EVALUATION OF EXTRACTION METHODS FOR METABOLITE PROFILING OF *APIS MELLIFERA* USING NMR SPECTROSCOPY

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Metabolite profiling provides an insight into a situation in a cell as metabolites represent status of the cell, tissue or whole organism. NMR, despite its relatively low sensitivity, offers high reproducibility, short acquisition time, possibility of unknown metabolite identification, and quantitative information. Honeybees (*Apis mellifera*), the most important insect in the world regarding the food production, suffer from major unresolved phenomena, like colony collapse disorder and overwintering problems, which threaten their economic utilization and food production chain as such.

In this study, we have developed and evaluated extraction protocols for NMR based profiling. We have evaluated different pre-extraction procedures –intestinal tract removal, separate head and body extraction and freeze drying, and five different extraction solvents plus five combinations. Proton NMR spectra (500 MHz) were processed using PCA analysis. Identification of compounds was based on comparison with the spectra of pure standards.

Inter-individual variations were negligible, but the intestinal tract removal was necessary. Freeze-drying as well as separate head and body extraction did not proved beneficial for the final results, and therefore are not recommended. Major amino acids, sugars and organic acids were identified. Comparison of their relative quantification in different solvents revealed that combination of water/acetonitrile 1:1 was superior in yield of most of the major compounds. On the other hand, water/methanol 1:1 showed better peak resolution despite the lower yield.

For NMR-based metabolomics the metabolite extraction is the most critical step. This study could contribute to clarification of phenomena such as colony collapse disorder or longevity of honeybees.